Ultrasonography of the fetal nose, maxilla, mandible and forehead as markers for aneuploidy

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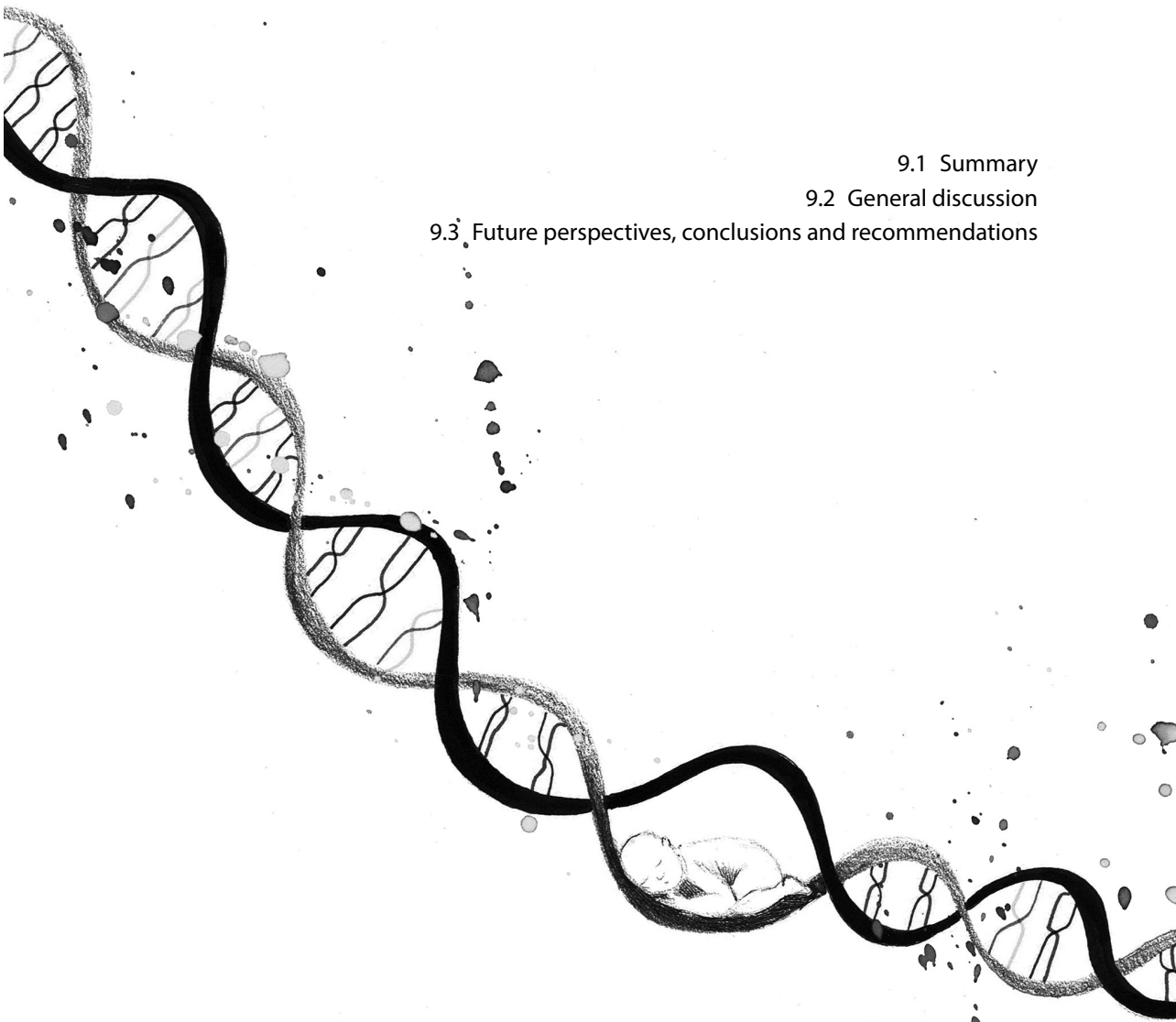
CHAPTER 9

Summary, general discussion and future perspectives



9.1 SUMMARY

Chapter 1 gives a brief insight in the history of ultrasound (US) as a tool to examine the fetus. US technique finds its roots in the early 1960's. The transition of US examination from a rudimentary technique, to the highly advanced screening and diagnostic tool that it is today is reviewed. Furthermore, the new development of three-dimensional (3D) US is discussed, with its merits and limitations compared to conventional two-dimensional (2D) US. Screening for Down syndrome (DS), the most common autosomal trisomy in life born infants is discussed. Also, an overview is given of the current screening options available in the first and second trimester. Special attention is paid to markers that can be found in the fetal profile during US examination in the second trimester. Furthermore, a brief introduction of Edwards syndrome (ES) is provided with information on incidence, major malformations observed during pregnancy and at birth, survival and screening possibilities.

In **chapter 2 and 3**, four structures located in the fetal profile, that were introduced recently as DS markers, are assessed in a group of euploid fetuses. Novel 3D based reference ranges are constructed. Subsequently, one of these markers, the prenasal thickness to nasal bone length (PT-NBL) ratio is tested in a small cohort of DS fetuses.

Chapter 2 assesses the feasibility of measurements of the nasal bone length (NBL), prenasal thickness (PT) and fronto-maxillary facial (FMF) angle, performed on the same 3D multiplanar corrected profile view in healthy second and third trimester fetuses. A three points scoring system was used to grade the images in terms of contrast and clarity. Only images with the highest two scores were used for further analysis. Measurements of each marker were repeated three times and the average was taken as the final measurement. It was significantly more often possible to achieve a high quality visualization of the NBL and PT (98% and 97%, respectively), than of the FMF angle (26%, $p < 0.001$). Both intra- and inter observer variability were superior in the first two markers. NBL increased significantly with gestation, from 3.3 mm at 15 weeks to 9.6 mm at 33 weeks gestation. PT was also correlated to gestational age, and increased from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks gestation. Reference ranges for both markers are presented. The FMF angle did not seem to be correlated to gestational age, but owing to the paucity of high quality FMF angle measurements, extensive analysis was not performed with this angle and no reference range was constructed. An interesting observation was that after we had redefined the measurement technique for NBL (carefully excluding the frontal bone from the measurement by measuring along the superior surface of the bone and not mid-way through), our reference range for the NBL showed a systematically smaller length than other 2D US based publications, whilst following the same curve. In conclusion, NBL and PT, measured on 3D rendered volumes, are easily applicable markers, whereas the FMF-angle is more challenging. Furthermore, care should be taken in excluding the frontal bone from the measurement.

In **chapter 3**, we have studied the ratio of the PT to NBL, the PT-NBL ratio, in normal and DS fetuses in the second and third trimester of pregnancy. The measurements of the study mentioned in chapter 2 were used to calculate the PT-NBL ratio in normal fetuses. The PT-NBL ratio did not

increase with gestational age (mean 0.61, 95% CI, 0.59 – 0.63; r = -0.04, P = 0.7). The 5th and 95th percentiles were 0.48 and 0.80, respectively. This reference range was used to compare to a small cohort of DS fetuses. The PT-NBL ratio was significantly higher in DS fetuses than in normal fetuses (P < 0.001) but was also stable throughout gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80; r = -0.35, P = 0.07). All DS fetuses had a PT-NBL ratio above the 95th percentile. When the 95th percentile of the PT-NBL ratio was used as a cut-off value, the detection and false positive rates for DS were 100% (95% CI, 89 – 100%) and 5% (95% CI, 2 – 11%), respectively. The positive likelihood ratio was 21.2. The conclusion of this study is that the PT-NBL ratio is a stable marker for DS in the second and third trimester of pregnancy. Most importantly, all DS fetuses in this series had a PT-NBL ratio above the 95th percentile, making it a very promising marker for DS.

Chapter 4 and 5 deal with the introduction of two markers for DS, the maxilla-nasion-mandible (MNM) angle and the fetal profile (FP) line, and assess four other markers for DS, the NBL, PT, PT-NBL ratio and prefrontal space ratio (PFSR). These markers are located in the fetal profile and aim to quantify the shape of the profile. The measurability and reproducibility of the MNM angle and FP line with its corresponding FP distance (the shortest distance between the FP line and frontal bone) is assessed in a group of euploid fetuses, **chapter 4** introduces them as markers for DS. This was done retrospectively in a cohort of 138 DS fetuses in the second and third trimester of pregnancy. Measurements were compared to our previously reported normal ranges. The MNM angle was significantly smaller in DS fetuses (mean 12.9º) than in euploid fetuses (mean 13.5º , p = 0.015). However, in only 16.9% of DS fetuses, the MNM angle was below the 5th percentile, although this was significantly more often than in euploid fetuses (p < 0.01). The MNM angle was not influenced by the gestational age (p = 0.48). Intra- and inter-observer reproducibility was expressed as intra-class correlation coefficient (ICC) with values of 0.89 and 0.61 for the MNM angle and 1.0 and 0.76 for the FP line, respectively. In the cohort of DS fetuses, none had a negative FP line. In the entire DS group, the FP line was positive in 41.1% of fetuses and correlated to both DS and gestational age (p < 0.001). As in a previously studied group of euploid fetuses, the FP line was never positive before 27 weeks gestation, we decided to divide the DS group in two cohorts: the second and third trimester. Their respective detection rates with false positive rates (FPR) were 28.4% with 0% FPR and 76.5% with 16.9% FPR for the second and third trimester, respectively. The FP distance increased with gestational age (p<0.001), with a mean distance of 3.1 mm. The FP distance was not significantly larger in DS fetuses than in euploid fetuses (p = 0.4). Small MNM angles were correlated with a positive FP line (p < 0.001). By means of this study we propose the FP line as a novel marker for DS with an extremely low false positive rate in the second trimester. As the FP line requires no reference values (as it is positive, negative or zero), its use is very easy.

In **chapter 5**, the four markers NBL, PT, PT-NBL ratio and PFSR are evaluated in a large cohort of second and third trimester DS fetuses. The markers were measured in 159 DS fetuses and compared to previously reported reference ranges. The median maternal age was 35.8 years, the median gestational age was $23^{+}1$ weeks. Intra- and inter-observer variability were best in NBL, PT and PT-NBL ratio, with intra-class correlation coefficients (ICC) of 0.98 and 0.93, 0.98 and 0.97, 0.94 and 0.92, respectively. The PFSR had ICC's of 0.67 and 0.67, respectively. NBL and PT were correlated to gestational age (p < 0.001), PT-NBL ratio and PFSR were not. All markers were correlated to DS

($p < 0.001$). The mean NBL, PT, PT-NBL ratio and PFSR were 5.08, 5.56, 1.26 and 0.34, respectively. The nasal bone was absent in 23 (15.4%) cases. In screening by all four markers, the PT-NBL ratio yielded the highest detection rate of 86.2%, followed by the PFSR (79.7%), PT (63.4%) and NBL (61.9%). In 33.6%, all markers were abnormal. In 4.7% of cases, all markers were normal. The combination of all four markers yielded the best detection rate of 95.3%, followed by a combination of PFSR and PT-NBL ratio with 93.8%. As the PT and NBL are used to calculate the PT-NBL ratio and PT is also used to calculate the PFSR, we were not surprised to find only the Multiple of the Median (MoM) NBL and PFSR to be independent of each other ($p = 0.10$). Both the DR for all markers as well as MoM NBL, MoM PT, PT-NBL ratio and PFSR, were not correlated to gestational age. In conclusion we propagate the use of the PT-NBL ratio and PFSR in the second trimester of pregnancy. Not only do these markers achieve a high detection rate of 94%, they are also very user-friendly in the fact that they require no knowledge of gestational age specific means.

Chapter 6 deals with the assessment of differences between measurements when using either 2D images or 3D volumes. Differences between six markers (NBL, PT, FP line, MNM angle, PT-NBL ratio and PFSR) when measured on 2D images (acquired with strict criteria) or 3D rendered volumes were analyzed, as well as their clinical application in screening settings. All six markers were measured in 105 datasets: 75 of euploid fetuses and 30 of DS fetuses. 2D images and 3D volumes were both derived separately in the same scanning session. The MNM angle measurements in 2D US were significantly larger by 1.0 degree ($p < 0.01$). In all other markers there was no significant difference between 2D or 3D US. Limits of agreement (LOA) for intra- and inter-observer variability were smaller in 3D measurements for all markers, except for the MNM angle. When the group of DS fetuses was analyzed separately, no statistical difference was found for any of the markers measured in 2D or 3D US, in their ability to discriminate between normal and DS fetuses. With this study, we have shown that, with exception of larger MNM angles in 2D images, no significant differences are found in a number of facial profile parameters and DS markers. In particular, NBL, PT, FP line, PT-NBL ratio and PFSR can be confidently used as DS screening markers in US examinations performed by 2D US, without missing out on the additional benefit of 3D US, provided the markers are measured in an as good as possible mid-sagittal image of the fetal profile.

Chapter 7 evaluates repeated measurements of the NBL, PT, nuchal fold (NF), PT-NBL ratio and PFSR in second and third trimester DS fetuses. Markers were studied retrospectively and compared to previously reported normal ranges. A total of 24 DS fetuses were analyzed. The median gestational age was 25 weeks. Median gestational age at initial examination was $20^{+}4$ weeks, and at final examination $29^{+}2$ weeks. The median interval between measurements was 39 days (range 14 – 98 days), with an average number of 2.6 visits per case. NBL, PT and NF increased significantly with gestational age ($p < 0.01$), PT-NBL ratio and PFSR did not. In 42% of DS cases, NF did not increase in at least one consecutive measurement, opposed to 4.8% and 13.6% for NBL and PT, respectively. The PT-NBL ratio was the most stable marker, remaining the same value in 95% of cases. In a 'mixed model' format, a corresponding trendline for repeated measurements was compared to the mean of euploid fetuses. In this format, the gestational age dependent markers (NBL, PT, NF) expressed more deviance with advancing gestation, but MoM values remained stable. The NF and PFSR showed the largest differences between measurements when every case was individually depicted. However for

the PFSR, most measurements were still far below the normal range. In this study we conclude that repeated measures of the NF and PFSR are the least likely to follow an expected and steady trend in a single DS fetus, probably due to challenging reproducibility. However, PFSR still remains a valuable marker, as most measurements are still far from normal.

In **chapter 8** we assess six markers that were initially introduced as markers for DS, as potential markers for Edwards syndrome (also known as trisomy 18) in the second and third trimester. The markers tested include the PT, NBL, PT-NBL ratio, PFSR, MNM angle and FP line. Measurements were compared to previously published normal ranges. In order to further investigate the clinical relevance of US markers for Edwards syndrome (ES), additional US findings (markers, structural anomalies, growth retardation) were noted, specifying whether they were detected at the initial routine 20-weeks scan or at the subsequent advanced US examination after referral for karyotyping. Fourty-three ES fetuses were included. Median maternal age was 37 years and median gestational age 21^{+2} weeks. As in DS, the NBL and PT were correlated to gestational age ($p < 0.001$), the other markers were not. The mean NBL, MNM angle, PT, PT-NBL ratio and PFSR were 3.76, 16.67, 4.25, 1.39 and 0.87, respectively. The FP line was zero (normal) in 53.7% of cases and negative (abnormal) in 46.3%. All markers were significantly correlated to ES. A short nasal bone was a prominent feature in ES fetuses, opposed to an enlarged PT. This was illustrated in the performance of the separate markers: in the detection rate for ES, the PT-NBL ratio yielded the highest detection rate (88.4%), followed by the NBL (83.7%), MNM angle (56.4%), FP line (46.3%), PT (27.9%) and the PFSR (20.5%). The false positive rate was 5%, except for the FP line, where it was 0%. Various combinations of the 4 best markers (NBL, FP line, MNM angle and PT-NBL ratio) yielded detection rates ranging between 90% and 95%. No structural anomalies were detected in 22% of fetuses at the initial scan and in 2% at the advanced scan. The main conclusions of this chapter are that the PT-NBL ratio and NBL are strong second and third trimester markers for ES. Furthermore, a negative FP line has a 0% false positive rate and the potential to differentiate between ES and DS, as in the latter the FP line is often positive. No major anomaly was observed at the initial scan in about 1 in 4 ES fetuses, underlining the role of second trimester facial marker evaluation.

Summary of the most important findings

Examination of markers in euploid fetuses:

- The PT, NBL and PT-NBL ratio are reproducible markers that are easy to measure.
- The FMF angle is often difficult to assess (in retrospect), as the landmarks which are used to construct this marker (palate, vomer) are often not clearly visible in the second and third trimester.
- It is important not to include part of the frontal bone when the NBL is measured, as our reference range showed a systematically smaller measurement, when compared to other publications.
- The PT-NBL ratio in euploid fetuses has a constant mean value of 0.61 throughout the second and third trimester. The 95^{th} percentile is 0.80.

Screening performance of markers in DS fetuses:

- The PT-NBL ratio yields the highest detection rate of 86.2%.
- The two best-performing DS markers in terms of detection rate, the PT-NBL ratio and PFSR, together detect 94% of DS fetuses and require no knowledge of a reference range.
- A positive FP line has an extremely low false positive rate in the second trimester of 0%.

Comparison of 2D and 3D US:

- When measurements in NBL, FP line, MNM angle, PT, PT-NBL ratio and PFSR are compared in 2D (required according strict criteria) and 3D images, only the MNM angle shows a small difference.
- No statistical difference was found between 2D and 3D acquired measurements for any of the six markers in their ability to discriminate between normal and DS fetuses.

Longitudinal analysis of DS markers:

- The reliability of NF as a second trimester DS marker is disputable.
- Repeated PFSR measurements in the same fetus are subject to considerable variation.
- The PT-NBL ratio is a very stable marker.

Screening performance of markers in ES fetuses:

- The PT-NBL ratio and NBL are strong second and third trimester markers for ES.
- A negative FP line showed a 0% false positive rate in this study and offers the potential to differentiate between ES and DS.
- No major structural anomaly was observed at the initial US examination in about 1 in 4 fetuses, opposed to 2% at the advanced US exam.

9.2 GENERAL DISCUSSION

This thesis has explored the potential of facial profile markers for identifying aneuploid fetuses in ultrasound (US) investigations performed beyond the first trimester.

The result of the thesis can be summarized as follows: of all markers that have been explored, the prenasal thickness (PT) to nasal bone length (NBL) ratio (PT-NBL ratio), is a strong second and third trimester US marker for both Down syndrome (DS) and Edwards syndrome (ES), whereas the fetal profile (FP) line is often positive in DS and negative in ES. For clinical practice, the great advantage of the PT-NBL ratio lies in the fact that the ratio is stable during pregnancy with the PT being about 2/3 of the NBL with the 95th percentile stable at 0.80. Moreover, this thesis demonstrates that, although 3D correction of the profile by 3D multiplanar mode allows definition of the correct midsagittal plane, the use of this correction is not essential when applying the markers in current clinical practice. This is an important issue, as it implies that the profile markers can theoretically be part of routine US investigation, even when the used US equipment does not include a 3D mode.

Of all the other studied facial profile markers in DS, the second best was the PSFR ratio. Its sensitivity was however slightly inferior to that of the PT-NBL ratio and the measurement might

be more time consuming. Moreover, it could be difficult to master and sensitivity in ES is low. The other markers, such as the PT and NBL (as separate markers) and the MNM angle, appear less effective. However, even if these markers do not seem to play an important role in the identification of DS and ES, the merit of this study is to have reinforced their use as instruments to study the fetal face. Familiarity with their use and application may be of great value when the ultrasonographer suspects an abnormal profile and needs this finding to be supported by an objective evaluation of facial proportions and relations. The less effective markers therefore still qualify as important instruments in the hands of the ultrasonographer, as they can be applied in the emerging field of fetal dysmorphology.

The studies included in this thesis were all retrospective. This has enabled inclusion of a large number of DS and ES fetuses, retrieved from databases of more than one centre. In case of a prospective study design, a lot of time would have been necessary to collect an equal number of cases. A clear limitation of a retrospective design – whereby cases are selected after the karyotype is known and the profile markers are measured on stored pictures – is that the sensitivity of the markers may be overestimated. However, with the rapid advent of cell free fetal DNA techniques in maternal blood as early screening for trisomies, we assume that the number of fetuses with trisomies at the second trimester scan may in the future be drastically reduced and therefore future validation of the data in prospective studies may become extremely unlikely.

A legitimate question regarding this study could be how it has been possible to collect so many cases of chromosomally abnormal fetuses reaching the second trimester undetected. This was possible, as many women in The Netherlands do not choose to undergo first trimester screening for aneuploidies. Later in pregnancy, they might be referred to a prenatal diagnostic center because of the finding of structural anomalies or other pathologies, such as growth retardation, detected at the second or even at a third trimester scan. This, in turn, prompted karyotyping before or after birth and from these cases, stored pictures of second and/or third trimester prenatal facial features of the chromosomally abnormal fetuses were retrieved. Moreover, the series of studies in this thesis should be seen as a logic continuation of the work our group started about ten years ago by applying the advantages of 3D US to the study of the fetal face[1]. These studies pointed out that 3D multiplanar mode technique could be of help in standardizing the planes for a morphometric evaluation of the fetal face. Conditions such as micrognathia, sloping forehead, bossing forehead, facial clefts etc. could be objectively measured[2-4]. In the first series of studies, we also reported for the first time on the detection of DS by using a combination of facial markers; the PT-NBL ratio[5]. In this study we reported a DR of 100% in 30 fetuses. This exceptionally high detection rate stimulated our group to focus on further studies concerning the application of all the previously defined facial markers in fetal trisomy screening. By extending the number of DS cases the performance of the PT-NBL ratio in the second study was, as expected, less than 100%. It is likely that, in a prospective design, this would become even lower. However, this ratio remains an exceptionally good marker for fetal trisomies in the second and third trimester and, if combined with other markers such as the PFSR, can reach detection rates up to 94%. This is better than all other previously known and used markers in the so called "genetic sonogram".

It is undisputed that for all kinds of practical and ethical reasons the preferred moment for screening for trisomies is in the first trimester. This is especially the case as the scenery of prenatal screening for trisomies is rapidly changing due to the introduction of non-invasive prenatal testing (NIPT). However, it is also true that for all kinds of other reasons it will never be possible to ensure that first trimester screening takes place in all pregnancies. Therefore it is very important that also in the second and third trimester of pregnancy, strong markers for trisomies are available. Participation in first trimester screening for trisomies in The Netherlands is low in comparison to other European countries, not reaching more than 30% of the pregnant population and even less in rural areas. A negative attitude towards termination of pregnancy (TOP) and acceptation of DS have been reported as reasons for the low uptake[6]. However, other factors, such as not being fully aware of the fact that even young women have a chance of having a DS baby or that by declining the CT a choice with clear consequences is being made, also play a role. A regrettable factor that may have influenced counselling and the attitude of women and care givers with respect to the CT is the fact that first trimester screening was free of charge until the end of 2014 only for women of 36 years and older. Unfortunately, in spite of pressure from various professional organizations, the Dutch Ministry of Health has decided to eliminate the inequality between older and younger women by establishing that all women, irrespective of their age, have to pay for the CT[7]. In case of increased risk, access to NIPT and invasive procedures will be free, whereas access to invasive procedures based purely on maternal age is not reimbursed anymore. The community of professionals involved in counselling and screening of pregnant women is anxious to see what the effects will be of such a new change in course of the Dutch policy makers. One may speculate that the uptake of the CT may decrease in general or increase only among older women, but the opposite may also occur, with more women choosing NIPT directly, irrespective of the Dutch regulations. Furthermore, we hope that the traditional first trimester scan will stay preserved in the future, as the goal of this scan is not only to screen for trisomies, but also for other anomalies. One way or another, we expect that for the time being, many cases of chromosomal anomalies will remain undetected until the moment of the 20-weeks scan which is part of routine prenatal care.

An ethical objection to screening for DS by means of the 20-weeks scan might be that most women undergo the scan with the expectation to see their baby, not realizing that it may also reveal unexpected malformations and even malformations related to chromosomal anomalies[8]. This was assessed by a Dutch study[9], indicating discordance between medical experts and pregnant woman's attitude towards the 20-weeks scan. The first group regarded the scan primarily as a mean to detect anomalies and, opposed to the pregnant women, considered the 20-weeks scan to have a similar value as first trimester screening for congenital anomalies. These findings raise the impression that women may not be sufficiently informed about screening for anomalies in general and not be fully aware of the implications for opting in or out. Another objection that has been raised is that women who decline the CT, also indirectly decline screening for trisomies at the 20-weeks scan. This suggests that women should be informed about the fact that detection of chromosomal anomalies, although less effectively, can also occur in the second trimester of pregnancy. Accordingly, one may even consider explicitly asking women whether they want specific measurements such as the NBL and PT to be carried out.

In conclusion, we consider the studies reported in this thesis as an important contribution in filling the gaps in the (Dutch) prenatal screening system and to provide measurement tools for objectifying fetal facial dysmorphology.

9.3 FUTURE PERSPECTIVES, CONCLUSIONS AND RECOMMENDATIONS

The fetal facial markers discussed in this thesis seem to be very promising adjuncts in prompting a strong suspicion of a chromosomal anomaly. Even when they are isolated, they can warrant further investigation. The results of this thesis are based on a retrospective analysis of prospectively collected data. This aspect, and the fact that examiners were not blinded to the karyotype, could be regarded as limitations of the study. To adequately confirm the findings of this thesis, a large prospective study would be necessary. This was also suggested by a recent meta-analysis on the nasal bone as a marker for aneuploidy. The authors Moreno-Cid et al[10] found retrospective studies to report structurally higher rates of abnormal nasal bones in Down syndrome (DS) fetuses than in prospective studies. When collecting data retrospectively, a selection bias in favour of including abnormal cases in the analysis is inevitable.

Most profile markers discussed in this thesis are clearly different in chromosomally abnormal fetuses. However, it is still unclear whether these markers, when isolated, can be used to discriminate between different chromosomal anomalies or if they can be used to identify other genetic syndromes. For instance, the PT-NBL ratio is enlarged in both DS and in Edwards syndrome (ES). On the other hand the FP line, influenced by a flat profile and retrognathia, can potentially discriminate DS from ES. Ideally it would possible to create an algorithm able to identify and discriminate different chromosomal anomalies and genetic syndromes, similarly to first trimester screening.

In the literature, several methods to quantify facial features are reported. The possibilities to draw lines and measure angles in the face are limitless, and the quest for the best marker is not over yet. The recently proposed idea of combining the fetal profile line and the mandibulo-maxillary line (of the PFSR) into a reverse MNM-angle is a good example of the evolution of existing markers into a sensitive clinical tool[11].

In this thesis, only fetuses of Caucasian parents were examined. For some markers like the nasal bone, it has already been established that ethnic variation influences the markers and their performance and should therefore be taken into account[12]. Further investigation of ethnic influences on all markers reported in this thesis is therefore recommended.

Ideally, we would hope that these relatively easy to use markers would be able to extend our insight in the pathophysiological mechanisms leading to facial dysmorphic features. It would also be desirable to establish a link with the severity of expression of the condition. If it would be possible to connect subtle anatomic variations like facial markers to postnatal outcome, it might be possible to offer a more specific prognosis in case of an affected pregnancy.

At this moment, the subject of intra-uterine dysmorphology is only at an early stage. The next big challenge will be, next to extending the diagnostic ability to identify more genetic syndromes,

to be able to use this diagnostic tool in earlier stages in pregnancy. This would allow more time for targeted genetic investigations where possible, and for parents to be optimally informed on the condition affecting their fetus, as to make a well informed decision concerning the future of the pregnancy.

Most markers assessed in this thesis, such as the PT-NBL ratio, are easily performed after simple training when a good profile view is obtained by 2D ultrasound. Therefore, we expect that in the future the measurement of this marker will become part of the routine 20-weeks scan, on condition that the mother wants to be informed about the likelihood of an aneuploidy. Finally, it is not unthinkable that these markers may already find an application in the first trimester of pregnancy.

Conclusions and recommendations

- 3D US enables visualization of the fetal face in utero.
- Typical facial features can be quantified in the profile view. 3D can be of help in defining the exact midsagittal profile view.
- The first trimester is the best moment for screening for aneuploidies. However not all women undergo first trimester screening. This means that cases of aneuploidies can still be identified at later scans
- The 'genetic sonogram' has recently been enriched by new fetal profile markers for aneuploidies.
- The most sensitive markers for second trimester DS screening are the PT-NBL ratio and the PFSR.
- The most sensitive marker for second trimester ES screening is the PT-NBL ratio.
- The FP line makes it easier to differentiate between DS and ES.
- Other markers investigated in this thesis are less prone to routine clinical application.
- Even in the event that non-invasive prenatal testing (NIPT) may transform the current aneuploidy screening policy, fetal dysmorphology will continue to exist and to expand. This thesis must be regarded as a further step in that direction.

REFERENCES

1. De Jong-Pleij EA, Ribbert LS, Tromp E, Bilardo CM. Three-dimensional multiplanar ultrasound is a valuable tool in the study of the fetal profile in the second trimester of pregnancy. Ultrasound Obstet Gynecol. 2010 Feb;35(2):195-200.
2. de Jong-Pleij EA, Ribbert LS, Manten GT, Tromp E, Bilardo CM. Maxilla-nasion-mandible angle: a new method to assess profile anomalies in pregnancy. Ultrasound Obstet Gynecol. 2011 May;37(5):562-9.
3. de Jong-Pleij EA, Ribbert LS, Pistorius LR, Tromp E, Bilardo CM.The fetal profile line: a proposal for a sonographic reference line to classify forehead and mandible anomalies in the second and third trimester. Prenat Diagn. 2012 Aug;32(8):797-802
4. de Jong-Pleij EA, Pistorius LR, Ribbert LS, Breugem CC, Bakker M, Tromp E, Bilardo CM. Premaxillary protrusion assessment by the maxilla-nasion-mandible angle in fetuses with facial clefts. Prenat Diagn. 2013 Apr;33(4):354-9.
5. De Jong-Pleij EA, Vos FI, Ribbert LS, Pistorius LR, Tromp E, Bilardo CM. Prenasal thickness-to-nasal bone length ratio: a strong and simple second- and third-trimester marker for trisomy 21. Ultrasound Obstet Gynecol. 2012 Feb;39(2):185-90.
6. Bakker M, Birnie E, Pajkrt E, Bilardo CM, Snijders RJ. Low uptake of the combined test in The Netherlands--which factors contribute? Prenat Diagn. 2012 Dec;32(13):1305-12.
7. Http://www.rivm.nl/Onderwerpen/D/Downscreening/Voorprofessionals/Wijzigingenper1januari2015. Accessed January 2015.
8. Ahman A, Runestam K, Sarkadi A. Did I really want to know this? Pregnant women's reaction to detection of a soft marker during ultrasound screening. Patient Educ Couns. 2010 Oct;81(1):87-93.
9. Schoonen HM, Essink-Bot ML, Van Agt HM, Wildschut HI, Steegers EA, De Koning HJ. Informed decision-making about the fetal anomaly scan: what knowledge is relevant? Ultrasound Obstet Gynecol. 2011 Jun;37(6):649-57
10. Moreno-Cid M, Rubio-Lorente A, Rodríguez MJ, Bueno-Pacheco G, Tenías JM, Román-Ortiz C, Arias A. Systematic Review and Meta-Analysis of the Performance of Second Trimester Nasal Bone Measurements in the Detection of Fetuses with Down Syndrome. Ultrasound Obstet Gynecol. 2013 Oct 21.
11. Hartge RD, Gembicki M, Weichert J. Interobserver variability in volumetric assessment of maxilla-mandibular-nasion complex integrity. .Zoek pagina in the abstract boek Poster presentation on the 24th world congress on ultrasound in obstetrics and gynecology.
12. Zelop CM, Milewski E, Brault K, Benn P, Borgida AF, Egan JF. Variation of fetal nasal bone length in second-trimester fetuses according to race and ethnicity. J Ultrasound Med. 2005 Nov;24(11):1487-9.